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Is Behavior or Morphology a More Sensitive Indicator of Central Nervous System Toxicity?

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Both behavior and morphology can be altered by exposure of the CNS to toxic substances. The brain is an organ with considerable structural redundancy and this presumably accounts for some of the ability of the CNS to maintain normal function in the presence of some structural damage. Compensation for damage may also occur through a form of "learning" due to the biochemical and morphological plasticity of the CNS. Examples of these kinds of compensation are enzyme induction and axonal sprouting. Compensatory changes such as these are likely to require days or weeks to develop. On the other hand, short-term, reversible effects of substances such as drugs are not likely to cause morphological changes at doses which affect behavior. The importance of appropriate quantitative data on both morphology and behavior in evaluation of the CNS toxicity of substances is evident.

Principles Relating Organ Structure and Function

Behavior offers a sensitive way to monitor damage to the functioning of the central nervous system. Recognition of this obvious fact as a general principle has been relatively recent, although it has been an established part of the toxic effects of certain chemicals for many years. Behavioral toxicology, as a term, has a recent origin as the principle just stated. It is the functioning of the central nervous system that is monitored by behavior, not damage for which compensation occurs or damage which affects only excess capacity or structural redundancy. All organs are equally subject to these considerations. Function may be the easiest phenomenon to measure in the intact organism, particularly when that organism is man, for whom invasive techniques are often impractical. Hence, tests which monitor function, if they are reliable and sensitive to damage, are desirable measures of toxicity. The inherent limitations of functional tests are those just stated: many organs possess excess capacity which can be damaged and go undetected by functional tests, and some organs can compensate for damage without permanent effects on function. Tolerance is a term which is used to describe compensatory mechanisms to effects of drugs on the CNS.

While one may wish to know if the functional capacity of an organ is diminished in any way by a toxic substance, it is hardly necessary to argue that a level of damage which is not detected by functional tests for the reasons stated above must still be considered damage. Loss of small amounts of irreplaceable or even replaceable tissue from a toxicant is significant partly because the fundamental principle of dose–response governs the effects of most toxicants. If dose X kills Y number of cells, then dose $X + X^1$ kills $Y + Y^1$ cells and so on until, at some increment of dose X, function is damaged.

One final principle which applies to the toxic effects of all chemicals on all tissues is that physical, chemical, and biological alterations underlie all functional alterations. It is a truism to state that no functional change occurs apart from changes in the chemistry of the tissue. The principles just outlined are summarized in Table 1.

Relatively brief, completely reversible effects are characteristic of single pharmacologic doses of drugs, and reversible pharmacologic effects may be impossible for the morphologist to detect. For

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Table 1. Principles of measurement of organ damage.

Organ damage can result in functional damage.
Homeostatic mechanisms can obscure functional damage.
Homeostatic mechanisms include structural redundancy and tolerance.
Chemical and physical changes in cells govern all functional changes.

example, morphologic changes in the kidney are not apt to be caused by diuretic drugs in doses which cause therapeutic effects. Diuretic effects are most readily seen by directly monitoring function of this organ. On the other hand, damage to kidney tubules, which is often slowly reversible or permanent, may be caused by toxic substances which are not diuretics and this type of damage is readily seen in histologic preparations of kidney tissue.

In the enthusiasm for the recently discovered importance of behavioral toxicology it has been proposed that, by monitoring the functioning of the central nervous system with behavioral tests, the ability to detect CNS toxicity will be improved over other types of tests because behavioral tests are more sensitive than other tests. This proposal is neither intuitively correct nor without basis and needs to be examined critically in order to achieve appropriate use of tests for CNS toxicity.

The central nervous system falls into the category of organs in which duplication or redundancy of structure exists, and in which compensatory mechanisms have repeatedly been demonstrated. Thus, it is one of the organs categorized in Table 1 as those for which functional tests have inherently limited sensitivity. Whenever a small fraction of a redundant structure is damaged or a compensatory mechanism is called into action, function will be normal until either kind of reserve capacity is exhausted. One might even say that the central nervous system is the classic example of such an organ.

Methods for Compensating for CNS Damage

Tolerance

Tolerance or adaptation of the central nervous system is a wide-spread phenomenon in pharmacology. It occurs in response to various effects of drugs. Biochemical changes associated with functional tolerance have been shown to develop in many areas where feed-back mechanisms exist which signal the altered state to cells. As a consequence, induction of various enzymes can occur. For example, evidence has recently been presented for drug-related enzyme induction for tyrosine hydroxylase (*1*) and dopamine-β-hydroxylase (*2*). The time scale for enzyme changes may vary from minutes to weeks depending on the drug dosage schedule and presumably several other factors (*3*). In this kind of tolerance, the behavior may return to normal as the enzyme changes, and the enzymatic change may persist during behavioral tolerance for as long as the drug is administered. The condition is reversible, and return of the enzyme level to normal follows removal of the drug. Some good examples of permanent tolerance or compensation to damage come from studies of experimental brain lesions. Many lesions produce temporary loss of function with recovery of behavior in days or a few weeks. A classic example is the septal lesion which produces a hyperreactive rat, but the duration of the effect is reduced by repeated handling, with recovery occurring in a few days. Otton and Gage (*4*) have proposed that there is a parallel return of brain catecholamines to control levels as the septal behavior disappears. Thus, tolerance or adaptation illustrate ways in which the brain can be damaged and still retain normal function.

Redundancy

Morphological reserves are particularly important in keeping function normal in the presence of morphological damage. When only a portion of a redundant system is damaged, function is unimpaired until the reserve potential of the system is exceeded. Redundancy may exist either when there is a larger-than-required pool of neurons or where alternate pathways are available. In either case, loss of a fraction of the neurons will not alter function. The embryology and morphology of the CNS indicate that there are pools of cells in which the individual cell is without unique properties, allowing normal function to continue in the presence of cell loss. However, there are more sensitive indicators of morphological changes in neurons than death of the cell.

Quite recently, paralleling the increased interest in behavioral toxicology, various investigators have turned to examination of the CNS with the light and electron microscopes. New techniques have been applied to the study of toxicants on the CNS, and some old ones, such as the Golgi stain, have been rescued from undeserved obsolescence. Thus, details of neuronal morphology are being examined and revealed in ways dramatically different from the traditional hematoxylin and eosin (H & E) stain used in histology. One area of great potential importance in the renaissance of morphology relates to the recognition of the plasticity of neuronal morphology. In the living organism, the neuron, long after the embryonic and postnatal period of differ-

entiation and maturation, retains a plasticity of shape clearly dependent on afferent connections. This phenomenon has been identified in a sufficient number of different types of neurons to achieve the status of a general principle of neuronal plasticity, although it is only fair to state that the bulk of neuronal types in the central nervous system have not been directly examined. Perhaps plasticity is a unique property of some types of neurons but it is, as a phenomenon, widely distributed throughout the CNS, from the spinal interneuron (*5*) to the pyramidal cells of the archipallium (*6*). The time frame over which neuronal plasticity in response to damage has been demonstrated ranges from a few days (*7*) to months (*8*). There is even the possibility that altered neuronal morphology is associated with learning (*9*) if the term "learning" is used in the broadest sense of the word. Thus, the potential of quantitative alterations in neuronal morphology for detection of subtle toxic effects on the central nervous system is sufficient to merit serious consideration.

Quantitative Methods in CNS Morphology

The necessity for quantification of morphologic changes is as real as the necessity in functional tests, i.e., behavior. In spite of methodological problems, quantification is as essential in methods which detect toxic effects on the nervous system as dose and duration are in exposure to a toxic substance. The ideal way to compare the relative value of tests which monitor function and those which detect morphologic changes would be to have dose–response and duration–response data for various tests and toxic substances. Then tabulation of the data as outlined in Table 2 would identify the degree to which the proposed compensatory and homeostatic mechanisms in the CNS prevent expression of toxicity in functional tests. There is, of course, the additional question of whether or not a given test of morphological or functional change is adequately sensitive. For example, examination of

Table 2. Response to toxic exposure.

	Pharmacologic effect (rapidly reversible)	Toxicologic effect (irreversible or slowly reversible)
	Time (hours-days)	Time (weeks-years)
Morphological response	No change observed	Permanent change
Functional response	Behavioral change	A. Permanent change B. Tolerance or adaptation (no change observed)

brain tissue with the light microscope with the use of stains for nucleic acids, such as toluidine blue, permits detection only of gross changes in cell structure, while other methods of morphological examination, such as electron microscopy, reveal more structural details. The same is true of functional tests. A 1-hr test of diurnal activity in rats may detect severe locomotor damage of some types, but may fail to detect nocturnal hyperactivity after brain damage (*10*). This type of specificity in regard to damage, either morphological or functional, is a limitation of all toxicologic tests. Only a portion of the organ's morphology or functional capacity is being examined in any one test. It is hardly likely that any test could be so global as to evaluate the total status of any organ, even one much simpler in structure and function than the CNS.

In Table 2, two alternatives are listed for toxicological effects on function: permanent change or tolerance. It can be asked if there might not be compensatory mechanisms involved in the CNS in all prolonged exposures to toxic substances. It is likely that compensatory mechanisms operate only in some systems. For example, tolerance to opiates develops much more readily to depressant than to excitant effects (*11*), and generally marked tolerance does not develop to excitant drugs. If there is no tolerance to some toxic effects, is morphology likely to be a more sensitive indicator of toxicity than behavior? It is only possible to state that there is very little direct evidence concerning the relative sensitivity of tests of structure or function. Such information is highly desirable, since there is currently much interest in tests which accurately reflect the state of the nervous system.

Before the relative sensitivity of morphological and functional tests can be evaluated, the types of tests which are available must be considered. As sophistication in behavioral testing has increased, so have morphological analytical methods. Development of operant conditioning offers a way to quantify some types of behavior, and ethological methods are increasingly quantified. This has been paralleled historically by the development of the electron microscope, which explores the structure of the neuron in great detail. In both functional and morphological studies of toxicity, the development of methods which quantify is of utmost importance. Five quantitative morphological methods which have been used successfully to detect CNS toxicity will be discussed. These are examples from the extensive literature which now exists on the structural effects of toxic chemicals on the CNS. The aim is to suggest types of structural damage which might be compared with functional tests. Types of damage

which have been found with morphological techniques are: cell loss, perikaryal shrinkage, axonal sprouting, reduced dendritic branching, and decreased axo-dendritic synapses.

There are several methods for counting the number of cells in a sample of brain tissue. In one of the more unusual experiments, Valverde (*12*) has shown by use of the Golgi stain that blinding or raising neonatal mice in the dark selectively reduces the number of dendritic branches in layers III and IV of the visual cortex. Reduction in cell number in a single layer of the cerebral cortex in a localized area of the cortex requires methods with considerable discrimination.

The size of the perikaryon was recorded by Klosovskii (*13*) as the product of the length and width of the cell body. Prenatal exposure of rats to anoxia reduced the size of the perikarya in several brain areas. In this investigation stains for axons or dendrites were not used and it is a little unexpected that a simple measurement of the cell body was adequate to detect a postnatal effect of prenatal anoxia.

Scheff and co-workers (*7*) have reported that a lesion of the entorhinal cortex causes sprouting of undamaged axons in the deafferented hippocampus, and a partial lesion preceding a second lesion by a few days accelerates the axonal sprouting. Thus, axonal plasticity has been proposed to be under some kind of quantitative control.

Dendrites of neurons in various parts of the brain have been shown to respond morphologically to both increased or decreased afferent contacts. In neurons with spiny dendritic processes, such as cortical pyramidal cells and Golgi type II interneurons, each spine bears a postsynaptic contact with an axon. Thus, counts of dendritic spines can be used to estimate the number of axodendritic synapses on a neuron. The technique of counting dendritic spines has been used in various experimental studies of brain lesions and responses to toxic substances, such as frontal cortical lesions (*14*), carbon monoxide exposure (*8*), and lead toxicity (*15*). Changes in spines have also been observed in pathological conditions in humans, such as mental retardation (*16*) and Alzheimer's disease (*17*). Dendritic branching has been shown to be reduced in experimental damage, for example, in transsynaptic degeneration of deafferented spinal neurons (*5*) and in spontaneous pathological conditions, for example, in epilepsy (*18*).

The combined power of the light and electron microscopes has shown clearly that the morphology of the neuron can be modified without death of the cell. The morphological changes may result from changes in the cell's environment through damage to afferents from other neurons or may be a result of changes within the cell. There is still a great deal to be learned by looking at details of cell structure. While some morphological changes can be subjected to quantification, as in the examples given here, it is unfortunate that few methods are available with which histochemical changes can be quantified in the light microscope. In order to understand causes of cell damage as well as detection of cell damage, quantification of histochemical techniques, such as those which identify some enzymes selectively, would be extremely valuable. One other relatively unexplored area of morphological sensitivity is the structure of the dendritic spines themselves. It has been proposed that the basis of learning may even reside in the plasticity of the neuronal synapse (*9*), and the dendritic spine is an interesting structure with which to examine this question. In 1970, Peters and Kaiserman-Abramof (*19*) described three different shapes of spines on the pyramidal neuron. The same types of spines have been seen on other spiny neurons, and these three types, plus a fourth, filamentous type, are sketched in Figure 1. Examples of the spine types can be seen in Figure 2. The filamentous type, not mentioned by Peters and Kaiserman-Abramof, has been seen in cases of mental retardation (*15*) and may represent a response to deafferentation or cell injury. Dendritic spines develop on neurons after migration in the late embryonic or neonatal period. In animals like the rat, which is born without spines on telencephalic neurons, the dendrites are short and along their length have many varicosities which gradually disappear as the dendrite lengthens and spines begin to form (*8*). According to Peters and Kaiserman-Abramof (*19*), the most common spine on the pyramidal neuron is the thin type (72%); 19% are stubby, and 9% are mushroom-shaped.

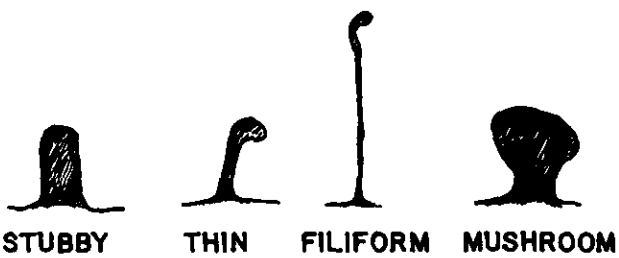

FIGURE 1. Types of dendritic spines found on cortical pyramidal cells and caudate interneurons, sketched from adult rat neurons stained with the rapid Golgi technique.

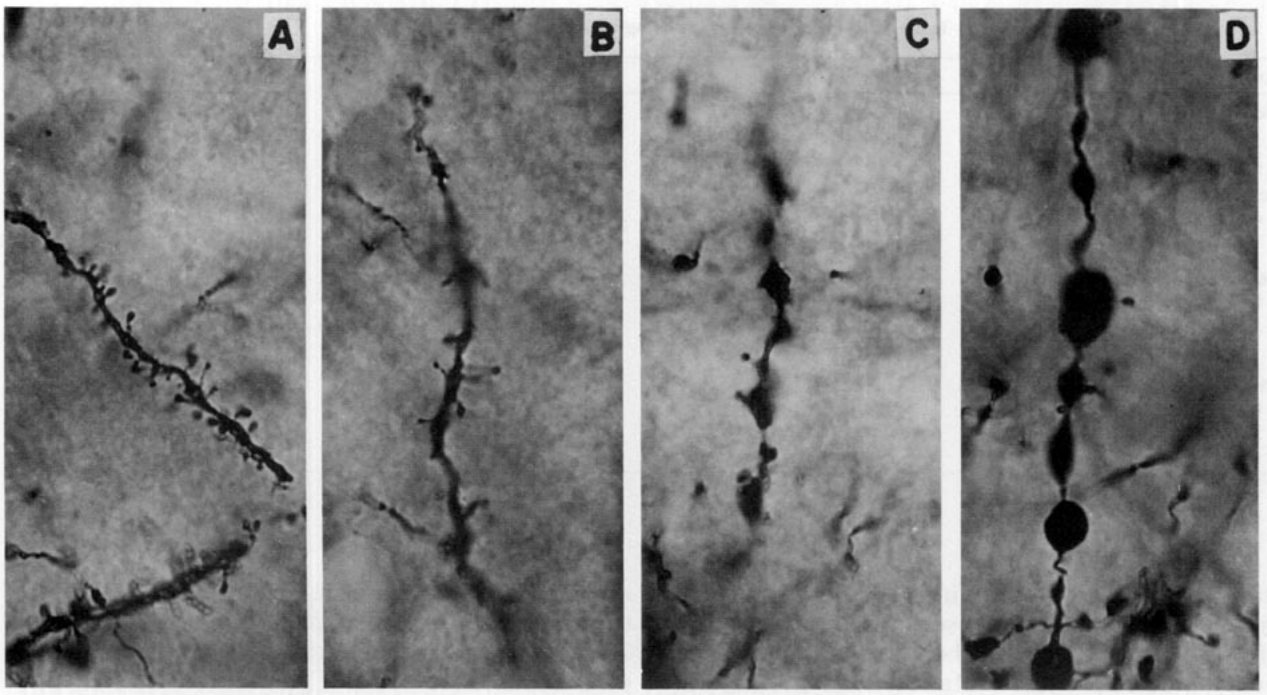

FIGURE 2. Dendrites of cortical pyramidal cells of 6-month-old rats: (*A*) dendrite from control rat showing four types of spines: stubby, thin, filiform, and mushroom-shaped; (*B*) Dendrite from control rat with fewer and more filiform spines than in (*A*); (*C*) Dendrite from rat irradiated on gestational day 15 (125 R) showing varicosities and few spines. (*D*) Dendrite from rat irradiated on gestational day 15 (125 R) showing large varicosities and almost no spines. Rapid Golgi technique; original magnification 1250 X.

This corresponds well with the distribution of the spine types found on the caudate interneuron in the 4 or 8-week-old rat (Table 3). In order to illustrate the potential value of such a detailed analysis, the following hypothetical argument is offered. If learning in the broad sense is related to dendritic spines, then compensatory mechanisms in the brain might involve increases in the number of spines in appropriate areas which are utilized in the development of compensation. Such changes have been reported in the caudate nucleus in recovery from carbon monoxide-induced hyperactivity (*8*, *20*) and in the auditory pathway after loss of vision (*21*). Further, if the different spine types represent stages of development of spines, then the number of each type should change in the learning process. No experiment testing this hypothesis is offered here, but indirect evidence is shown in Figure 3. If the most common spine in the mature neuron can be expected to be the mature spine, then the thin type should be the mature spine. The type which shows an early developmental peak should be the immature spine, and the stubby type fits this criterion (Fig. 3). Thus, it might be possible to detect a learning process by examination of dendritic spine types. This argument is not convincing at this time because adequate data are not available, but the argument does indicate that morphology may have much to offer in the study of dynamic processes in the CNS.

Parallel Morphological and Behavioral Studies

The final question remains of experimental data on the roles of morphology and behavior as

Table 3. Development of spines on caudate interneurons.

Postnatal age, weeks	Number of dendrites	Spine type, %				Spines per 10 μm (range)
		Filiform	Thin	Stubby	Mushroom	
1	25	56	25	12	6	1.6 (0-2)
2	19	18	54	26	2	12.5 (5-16)
4	20	6	70	21	4	18.8 (7-23)
8	17	10	73	11	8	20.3 (9-25)

Table 4. Comparison of morphological and behavioral damage.

Toxic substance	Morphology	Behavior	Reference
Methylmercury			(22)
10 μg	Focal Purkinje cell loss	None	
50 μg	General Purkinje cell loss	Ataxia	
Lead acetate			(15)
400 mg/kg	Parietal pyramidal cell loss of spines	Learning deficit	
Gestational x-irradiation (125 R)			(23)
6 weeks	None	None	
5 months	Cortical pyramidal cell decreased spines	Hyperactivity	
Carbon monoxide			(8)
6 weeks	None	Hyperactivity	
5 months	Caudate interneuron increased spines	None	

indicators of CNS toxicity. While the logical argument is easy to propose, critical experiments are rare in which careful behavioral studies have been paired with detailed morphological examination. Recently, some experiments have been performed which are directed toward detailed examination of both structure and function. Four examples are listed in Table 4. In two of these, behavioral changes were not reported at doses or times when morphological changes were seen. In the methylmercury study by MacDonald and Harbison (22), focal areas of Purkinje cell loss in the cerebellum were seen at low doses, and in the carbon monoxide experiment (8) the altered dendritic spine counts occurred in animals displaying normal locomotion. The possibility that behavior was altered in a way not measured, exists in both cases. In the study of neonatal lead acetate by Zenick and co-workers (15), both behavior and cortical pyramidal cells showed changes at the dose employed. Clearly more data are needed. Finally, parallel change in structure and function can be seen in some effects of prenatal x-irradiation. When rats are subjected to whole body irradiation with 125 R on day 15 of gestation, the activity of these animals is normal in the postnatal period until they mature. This is true of exploratory behavior (23) and nocturnal behavior in a residential maze (Table 5). As the delayed behavioral change occurs, the neurons of the cerebral cortex also develop altered morphology. This consists of an increase in the number of dendritic varicosities and loss of spines so that some of the cortical neurons begin to resemble the immature neuron (Fig. 2). Some

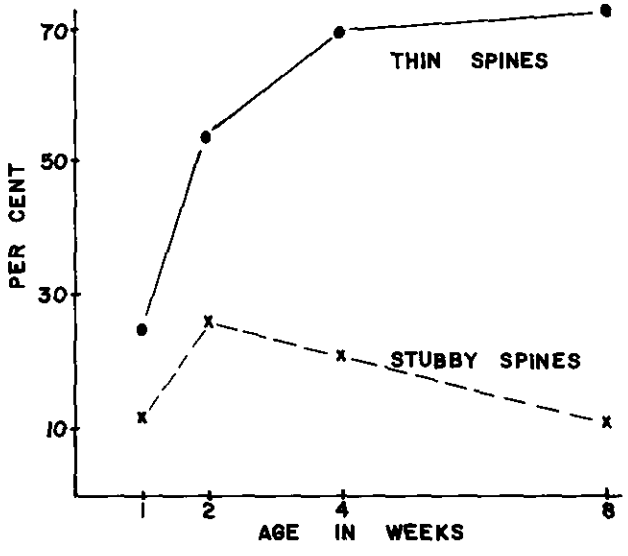

FIGURE 3. Development of thin and stubby spines on caudate interneurons in rats from 1 to 8 weeks postnatally: (•) thin spines; (x) stubby spines. Spines visualized with rapid Golgi stain.

Table 5. Nocturnal activity in rats after gestational x-irradiation.

	Photocell counts per hr	
Age of rats	Control	Irradiated
6 weeks	484 ± 63	425 ± 57
5 months	374 ± 28	620 ± 55[a]

[a] $p < 0.05$.

neurons in control rats also show these changes, but the percentage is higher in irradiated rats.

It should be no surprise that behavior alterations can at times be shown to parallel structural alterations and this principle is stated in Table 1. What is needed is careful evaluation of both kinds of studies in order to obtain the most insight into the action of toxic substances on the CNS. Awareness of the inherent limitations of functional tests discussed above should not obscure their vital role in understanding and evaluating the central nervous system.

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